Network Analysis of MICRORNAS Genes and Their Regulation in EWING’S Sarcoma

Kun Xie, Xiaoxin Guo, Kunhao Wang, Minghui Zhu, Shang Wang
Symbol Computation and Knowledge Engineer of Ministry of Education, Department of Computer Science and Technology, Jilin University, Changchun, Jilin, P.R. China

ABSTRACT: Experimentally validated data of the genes, microRNAs (miRNAs) and transcription factors (TFs) that affect EWS can be found in many literatures and databases. However, majority of them are in scattered form. The systematic regulatory mechanism has yet to be revealed. We constructed three regulatory networks hierarchically, including differentially expressed network, related network and global network. The differentially expressed network can be used as a faulty map of EWS. If the errors can be corrected, the cancer may be prevented and even cured in theory. Next, we compared their similarities and differences, a number of significant pathways, some self-adaptation associations and circle-regulations were highlighted. The differentially expressed network illuminated the pathogenesis of EWS. In addition, the related network further delineates the regulatory mechanism related with EWS. This paper systematically presented the regulatory mechanism of EWS and supplied gene therapy researchers with theoretical basis to concentrate on crucial genes and miRNAs.

1 INSTRUCTIONS

EWS is a malignant disease that mainly appears in children and adolescents. Survivors of EWS have the lowest health quality of life scores and high expenditure for treatment (Nakatani, F. 2012.). Experimentally validated data demonstrated that differentially expressed genes and differentially expressed miRNAs play an important role in many biological processes in EWS, so did the related genes and related miRNAs.

TFs are prominent regulators for the expression of genes (Hobert, O. 2008.). They are certain kinds of proteins that can activate or repress transcription of genes by binding to upstream regions of genes. Normally, they regulate gene expression alone or sometimes co-regulate with other proteins.

MiRNAs are 21-24 nt non-coding RNAs. They target at genes to regulate extensive biological processes. Numerous databases, including computationally predicted methods (Betel, D. 2008.) and experimentally validated databases (Papadopoulos, G.L. 2009.), supply enough resource to study relations between miRNAs and their targets.

Host genes are those genes where miRNAs locate. Researchers have found that the transcription of miRNAs and their host transcripts are concurrent and identified exonic and intronic (Rodriguez, A. 2004.). Intronic miRNAs and their host genes usually coordinately express and act as a potential partner in achieving biological function and influence the modification of pathways (Cao, G. 2010.).

Molecular biologists and medical researchers have conducted an amount of experiments in the field of EWS and believed that cancer is caused by differentially expressed genes and differentially expressed miRNAs. However, these experiments largely concentrated on a single element only, gene or miRNA, resulting in a situation that it is difficult to understand the general pathogenesis of EWS on the whole. In this paper, all the elements in EWS are taken into consideration rather than several of them. miRNAs, their targets, their host genes, and TFs were investigated to recognize those momentous pathways of EWS. Three kinds of relationship between the elements in EWS will help in understanding their control mechanisms in EWS, which are miRNAs locating on host genes, genes regulating miRNAs and miRNAs targeting at target genes. Experimentally validated dataset of human miRNAs and their target genes were collected from literatures and databases. Furthermore, a number of predicted TFs were obtained by the P-match method and were considered to be EWS-related genes. Three regulatory networks were created on the basis of the relationship we obtained according to the degree of correlation between the elements and EWS, the three networks are differentially expressed network, related network and global network. All the experimentally validated relationships formed global network at the time of writing. Considering the
complexity of constructing all pathways related with EWS, regulatory relationships of differentially expressed elements and predicted TFs were extracted to complete the pathways. Among the three networks, the differentially expressed network can be applied as a fault map when EWS emerges. Meanwhile, the related network and global network are used as an assistive tool. If the errors in differentially expressed network can be corrected, the cancer may be prevented and even cured in theory. The differences and similarities are significant meaningful in discovering important element and pathway of EWS, so our study concentrated on them and made necessary comparing.

2 MATERIAL AND METHODS

2.1 Material collection and data processing

In this paper, experimentally validated dataset of human miRNAs and their target genes were collected from the Tarbase 5.0 and miRTarBase. Official symbols from National Center for Biotechnology Information (NCBI) database are used for unifying each gene and miRNA.

The experimentally validated dataset of human TFs and the miRNAs regulated by them were gathered from TransmiR (Juan, W. 2009.), which is a manually extracted dataset describing relationships between TFs and miRNAs.

The host genes of human miRNAs were extracted from miRbase (Kozomara, A. 2011.) and NCBI. Similarly, this paper used official symbols and IDs to unify each host gene.

Differentially expressed genes in EWS were collected from Cancer Genetics Web, NCBI SNP database (http://www.ncbi.nlm.nih.gov/snp/) and pertinent literatures.

So does the EWS-related genes, and EWS-related genes were also collected from GeneCards database (Marilyn, S. 2010.) and pertinent literatures including those genes which affect tumor growth, migration, radial therapy and clinical outcome of EWS. So the differentially expressed genes are included in related genes.

Furthermore, our study obtained the popular TFs by P-match method (Chekmenev, D.S. 2005.) and those TFs were considered as EWS-related genes and only focused on these TFs that appear in transmiR. We downloaded 1000nt promoter region sequences of the targets of differentially expressed genes from UCSC database (Fujita, P.A. 2011.). P-match method, which combines pattern matching and weight matrix approaches, was applied to identify transcription factor binding sites (TFBSs) in 1000 nt promoter region sequences and TFBSs were mapped upon the promoter region of targets. Matrix library is also sets of known TF-binding sites collected in TRANSFAC, so it provides the possibility to search for a large variety of different TF binding sites.

This paper used the vertebrate matrix and restricted high quality criterion for matrix and extracted differentially expressed miRNAs from pertinent literatures and mir2Disease (Bao, J. 2012.), which is a manually created database about differentially expressed miRNAs in various human diseases. EWS-related miRNAs were manually collected from permanent literatures.

3 THREE NETWORKS CONSTRUCTION

Three regulatory networks of EWS were constructed, which are differentially expressed network, EWS-related network and global network.

It is inferred that the differentially expressed genes and differentially expressed miRNAs in transcription process may result in cancer. We extracted differentially expressed elements (genes, miRNAs and TFs) as well as relationships among them from the global network to construct the differentially expressed network. It is regarded as a core network in this paper.

Similarly, this method was used in generating EWS-related network. It is quite natural that the differentially expressed network is apart of the EWS-related network. The additional pathways of related network are possible in causing cancer. The EWS-related network is regarded as an assist in further research of the transcription mechanism of EWS.

All regulatory relations on host genes, target genes, miRNAs and TFs were extracted from aforementioned data and generate a network. After combining all these relationships, our study got the global regulatory network, which contains the differentially expressed network and related network.

4 RESULTS

4.1 Differentially expressed network of EWS

Figure 1 displays the significant regulatory relationships between differentially expressed elements in EWS.

This paper regarded TFs as a kind of special gene, So TFs, host genes and target genes are presented as round, and miRNAs are diamonds. Delta arrow represents a TF regulating a miRNA. Arrow in T shape represents a miRNA targets a gene (target gene is located in the T side). Arrow with a solid dot represents the relationship of a miRNA and its host gene (host gene is located in the solid dot side).
This network consists of two TFs, TP53 and FLI1, 39 miRNAs, their targets and host genes. Those elements are all differentially-expressed except for the host genes. Fig. 1 shows three types of relationships between each pair in EWS, which are miRNAs targeting at target genes, TFs regulating miRNAs and host genes including miRNAs.

![Figure 1. Differentially expressed network of gene and miRNA in EWS.](image)

And the significance of the TFs related pathways in Figure 1 obviously is there for EWS. There are 4 miRNAs target TP53 which regulates 8 miRNAs, hsa-miR-145 targets FLI1 which regulates 3 miRNAs. It is noteworthy that TP53 regulates hsa-miR-145 which targets FLI1, TP53 regulates hsa-miR-29a, hsa-miR-29b, hsa-miR-29c that target BCL2, FLI1 regulates hsa-let-7a-1, hsa-let-7a-2, hsa-let-7a-3 that also target BCL2, so it suggests that TP53 could indirectly influence the expression of BCL2 and FLI1, TP53 and FLI1 could indirectly influence the expression of BCL2. Besides, a self-adaptation relationship was identified between TP53 and hsa-miR-125b-1, TP53 and hsa-miR-125b-2.

BCL2 is the founding member of the Bcl-2 family of regulator proteins that regulate cell death, by either inducing (pro-apoptotic) or inhibiting it (anti-apoptotic). So BCL2 plays an important role in EWS. In pediatric EWS a chromosomal translocation generates a fusion of the 5’transactivation domain of EWS with the 3’Ets domain of Fli-1. The resulting fusion oncoprotein, EWS/Fli-1, acts as an aberrant transcriptional activator. So FLI1 is very important in EWS. There are some other genes that are targeted by some miRNAs but do not regulate any miRNAs and miRNAs that target some genes but none genes regulate them, theses elements that are the end or head of regulatory pathways and TFs should be paid much attention to in research.

Besides, we also noticed some special features between miRNAs and their host genes in Fig. 1. A host gene may include one or several miRNAs targeting other genes. For example, C21orf34 has two miRNAs hsa-miR-125b-1 and hsa-miR-125b-2, which target CDKN2A and TP53. CDKN2A is frequently mutated or deleted in a wide variety of tumors, and is known to be an important tumor suppressor gene. A miRNA may locate in one or several genes. For example, hsa-miR-92a-1 located in C13orf25 and M2R17HG.

We can conclude that the differentially expressed genes and differentially expressed miRNAs act as significant biological function in EWS. The differentially expressed network shows some information about expression level of differentially expressed miRNAs in EWS. The abnormally expressed network revealed the pathogenesis of EWS in a way.

4.2 Related network of EWS

EWS-related network contains 67 related genes, 93 related miRNAs and their targets. In this paper, differentially expressed network is included in related regulatory network of EWS, while related network shows more complex regulatory relations than differentially expressed network.

The network shows additional pathways of genes and miRNAs. In related network, TFs E2F1, MYC, NFkB1, NXX2-5, TP53, ESR1, TGFB1 regulate more miRNAs expression and they can be more influential with other factors. For instance, E2F1 regulates hsa-let-7a (7a-1, 7a-2, 7a-3), hsa-let-7a targets E2F2, IGF2, MYC, NFkB1, BCL2, CASP3, CASP8, CASP9 and E2F1. So E2F1 and hsa-let-7a, NFkB1 and hsa-let-7a-3 form self-adaption relationships. This kind of relationship can gain...
MYC regulates 27 miRNAs. These miRNAs target 37 related genes including MYC, ESR1 and so on. In turn, hsa-miR-18a, hsa-miR-19a, hsa-miR-19b-1, hsa-miR-19b-2, hsa-miR-20b, hsa-miR-22, hsa-miR-221 target TP53, which highlights the importance of MYC.

Current research has found different biological functions of different genes. The TGFB1 gene provides instructions for producing a protein called transforming growth factor beta-1 (TGFβ-1). The TGFβ-1 protein helps control the growth and division (proliferation) of cells. The overactive TGFβ-1 likely disrupts the regulation of bone growth and impairs muscle and body fat development. While disruption in the regulation of TGFβ-1 activity can lead to increased bone density and other features of EWS. So we should pay much attention to TGFB1 and the miRNAs that regulate TGBF1 because its mutation has significant corresponding relation with the clinical feature of EWS (www.ghr.nlm.nih.gov/gene/).

Comparing with the differentially expressed network, the related network extends the regulatory relationships of EWS and the new additional pathways of related network may partially illuminate the emergence regulatory pathways of EWS and some important factors with significant corresponding relation with the clinical feature of EWS.

4.3 Global network of EWS

This network revealed more complicated interactive relationships than related network of EWS. Obviously, the global network includes differentially expressed network and related network. So there are more TFs, miRNAs as well as their targets and their host genes than related network. It is an experimentally validated biological network in the human body.

4.4 Host genes and their miRNAs of EWS

In current study, host genes were considered as differentially expressed genes in EWS when their miRNAs are differentially expressed. They also influence EWS’s procession. Figure 2 reveals important relationships between miRNAs and their hosts. Hsa-let-7a-1, hsa-let-7a-2 and hsa-let-7a-3 locate in MIRLET7A1 and these entire three miRNAs target BCL2, E2F1, and NFKB1. At the same time, they form different self-adaption relationship. And hsa-miR-18a, hsa-miR-19a, hsa-miR-19b-1, hsa-miR-19b-2, hsa-miR-20b, hsa-miR-22, hsa-miR-221 target ESR1, and hsa-miR-221 targets TP53, which highlights the importance of MYC.

All the four miRNAs take BCL2 as their target gene. This paper will not list all relationships one by one. Host genes are important for the determination of relationships linking to specific miRNAs and may contribute to understanding of EWS.

4.5 Transcriptional network of predicted TFs

Figure 3 includes thirteen differentially expressed miRNAs, which are regulated by predicted TFs. The predicted TFs are got by P-Match method. That is, 1000-upstream sequences are imported into the software to obtain the predicted TFs which are also included in related genes. These differentially expressed miRNAs and predicted TFs construct a transcriptional network and show the regulatory relationships in EWS. TFs and miRNAs orderly
influence the expression of their targets or the elements regulated by them.

Through the analysis of figure 3, our study can come to some conclusions. A differentially expressed miRNA may be regulated by more than one TF, for example, hsa-miR-29a can be regulated by YY1 and NFKB1. A TF can be the targets of more than one differentially expressed miRNA, for example, YY1 can be targeted by hsa-miR-34a and hsa-miR-31.

A TF may indirectly affect other TFs with the aid of differentially expressed miRNAs. In addition, one differentially expressed miRNA may indirectly affect other miRNAs with the aid of TFs, for example, NFKB1 regulates hsa-miR-125b-1 and hsa-miR-125b-2, hsa-miR-125b-1 and hsa-miR-125b-2 targets E2F3, and E2F3 regulates hsa-let-7a (7a-1, 7a-2, 7a-3), which target NFKB1. E2F1 form 4 self-adaption relationships with hsa-let-7a (7a-1, 7a-2, and 7a-3) and hsa-miR-106a.

We can conclude that a TF may regulate one or more differentially expressed miRNAs and a differentially expressed miRNA may target at one or more TFs. A TF indirectly influences another TF by some differentially expressed miRNAs and an abnormally expressed miRNA indirectly influences another miRNA by some TFs. Figure 3 can contribute further research in regulatory mechanism of EWS.

4.6 Regulatory relationships of differentially-expressed gene

For the purpose of providing a clear description, this paper classified and listed those neighbor nodes according to regulatory relationships in three levels networks. Those nodes consist of TFs gained by using P-match method, differentially expressed genes and miRNAs. The successor and the precursor nodes of the differentially expressed genes in forgoing networks were extracted to compare and analyze those regulatory pathways. Among these genes, two genes (FLI1 and TP53) show a special feature that each gene regulates the miRNA, and it is targeted by miRNA(s).

We firstly focused on the TFs.

In table 1, 4 miRNAs target TP53 and TP53 regulates 8 miRNAs in differentially expressed network, 4 miRNAs target TP53 and TP53 regulates 11 miRNAs in related network, 13 miRNAs target TP53 and TP53 form self-adaption relationships in all three networks; they are all differentially expressed, which means they are crucial in EWS. In this respect, FLI1 is similar with TP53.

Secondly, this type of TF consists of four kinds of neighbors (three kinds of precursor nodes, one kind of successor node), including SOX2 and CDKN2A. Hsa-miR-145 targets SOX2 while SOX2 regulate no miRNA in differentially expressed network and related network.

Next, we concentrate on the rest genes with no miRNAs to regulate. In the differentially expressed network, the only type of genes that is not TF consists of three kinds of neighbors (three kinds of precursor nodes). Such as, BCL2 and TGFBR2 which are targets of several miRNAs, but have no miRNA to regulate. It is suggested that they may be the last node in the pathway.
Table 1. Regulatory relations between TP53 and miRNAs.

<table>
<thead>
<tr>
<th>Differentially expressed network</th>
<th>Related network</th>
<th>Global network</th>
<th>Gene symbol</th>
<th>Differentially expressed network</th>
<th>Related network</th>
<th>Global network</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-125b-1</td>
<td>miR-125b-1</td>
<td>miR-125b-1</td>
<td>miR-125b-1</td>
<td>miR-125b-1</td>
<td>miR-125b-1</td>
<td>miR-145</td>
</tr>
<tr>
<td>miR-125b-2</td>
<td>miR-125b-2</td>
<td>miR-125b-2</td>
<td>miR-125b-2</td>
<td>miR-125b-2</td>
<td>miR-125b-2</td>
<td>miR-34a</td>
</tr>
<tr>
<td>miR-221</td>
<td>miR-221</td>
<td>miR-221</td>
<td>miR-145</td>
<td>miR-145</td>
<td>miR-107</td>
<td>miR-125b-1</td>
</tr>
<tr>
<td>miR-222</td>
<td>miR-222</td>
<td>miR-222</td>
<td>miR-29a</td>
<td>miR-29a</td>
<td>miR-29a</td>
<td>miR-125b-1</td>
</tr>
<tr>
<td>miR-125a</td>
<td>miR-125a</td>
<td>miR-125a</td>
<td>miR-29b-1</td>
<td>miR-29b-1</td>
<td>miR-29b-1</td>
<td>miR-125b-2</td>
</tr>
<tr>
<td>miR-1285-1</td>
<td>miR-1285-1</td>
<td>miR-1285-1</td>
<td>miR-29b-2</td>
<td>miR-29b-2</td>
<td>miR-143</td>
<td>miR-145</td>
</tr>
<tr>
<td>miR-1285-2</td>
<td>miR-1285-2</td>
<td>miR-1285-2</td>
<td>miR-29c</td>
<td>miR-29c</td>
<td>miR-145</td>
<td>miR-145</td>
</tr>
<tr>
<td>miR-15a</td>
<td>miR-15a</td>
<td>miR-15a</td>
<td>miR-34a</td>
<td>miR-34a</td>
<td>miR-215</td>
<td>miR-215</td>
</tr>
<tr>
<td>miR-16-1</td>
<td>miR-16-1</td>
<td>miR-16-1</td>
<td>miR-107</td>
<td>miR-107</td>
<td>miR-29a</td>
<td>miR-29a</td>
</tr>
<tr>
<td>miR-25</td>
<td>miR-25</td>
<td>miR-25</td>
<td>miR-143</td>
<td>miR-143</td>
<td>miR-29b-1</td>
<td>miR-29b-2</td>
</tr>
<tr>
<td>miR-30d</td>
<td>miR-30d</td>
<td>miR-30d</td>
<td>miR-215</td>
<td>miR-215</td>
<td>miR-29b-2</td>
<td>miR-29b-2</td>
</tr>
<tr>
<td>miR-612</td>
<td>miR-612</td>
<td>miR-612</td>
<td>miR-29c</td>
<td>miR-29c</td>
<td>miR-155</td>
<td>miR-155</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>miR-192</td>
<td>miR-192</td>
<td>miR-194-1</td>
<td>miR-194-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>miR-194-2</td>
<td>miR-194-2</td>
<td>miR-200a</td>
<td>miR-200a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>miR-200b</td>
<td>miR-200b</td>
<td>miR-200c</td>
<td>miR-200c</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>miR-200c</td>
<td>miR-200c</td>
<td>miR-34b</td>
<td>miR-34b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>miR-34b</td>
<td>miR-34b</td>
<td>miR-34c</td>
<td>miR-34c</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>miR-34c</td>
<td>miR-34c</td>
<td>miR-519d</td>
<td>miR-519d</td>
</tr>
</tbody>
</table>

4.7 Regulatory relationships about differentially expressed miRNAs

This paper compared and analyzed each differentially expressed miRNA using the same method. Among these miRNAs, 13 differentially expressed miRNA and corresponding genes form 17 self-adaption relations.

Firstly, this type of miRNA consists of six kinds of neighbors (three kinds of precursor nodes, three kinds of successor nodes), including eleven miRNAs. Table 2 shows hsa-let-7a-1, its precursors and successors as well as their regulatory relationships. It has been demonstrated that direct repression of let-7a by EWS-FLI-1 participates in the tumorigenic potential of ESFT cells in vivo (Bao, J. 2011.). FLI1 regulates hsa-let-7a-1 that targets BCL2 in the differentially expressed network. There are four genes regulate hsa-let-7a-1 that targets eight genes in the related network. There are five genes regulate hsa-let-7a-1 that targets 43 genes in the global network. So we can conclude that FLI1 may influence BCL2 indirectly. E2F1 and MYC form two kinds of self-adaption relationships with hsa-let-7a-1. Hsa-let-7a-1 targets MYC which regulates 27 miRNAs. Hsa-miR-145 targets FLI1 which regulates hsa-let-7a-1.

Hsa-let-7a-1 also indirectly influences other miRNAs by some TFs, and in turn, some miRNAs also indirectly influence hsa-let-7a-1 by some TFs.

Secondly this type of miRNA consists of five kinds of neighbors (two kinds of precursor nodes, three kinds of successor nodes), including seven miRNAs. For example, hsa-miR-181b-1, hsa-miR-221.

Thirdly, this type of miRNA consists of four kinds of neighbors (one kind of precursor node, three kinds of successor nodes or two kinds of precursor nodes, two kinds of successor nodes), including nine miRNAs. For example, hsa-miR-181a, hsa-miR-23a, hsa-miR-27a.

Fourthly this type of miRNA consists of three kinds of neighbors (one kind of precursor node, two kinds of successor nodes or three kinds of successor nodes), including two miRNAs (hsa-miR-181d and hsa-miR-31).

Table 2. Regulatory relations between hsa-let-7a-1 and genes.

<table>
<thead>
<tr>
<th>Differentially expressed network</th>
<th>Related network</th>
<th>Global network</th>
<th>miRNA symbol</th>
<th>Differentially expressed network</th>
<th>Related network</th>
<th>Global network</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLI1</td>
<td>FLI1</td>
<td>FLI1</td>
<td>BCL2</td>
<td>BCL2</td>
<td>BCL2</td>
<td>KIT</td>
</tr>
<tr>
<td>E2F1</td>
<td>E2F1</td>
<td>let-7a-1</td>
<td>E2F1</td>
<td>BCL2</td>
<td>BCL2.1</td>
<td>MCL1</td>
</tr>
<tr>
<td>E2F3</td>
<td>E2F3</td>
<td></td>
<td>E2F2</td>
<td>BCL2.1</td>
<td>MUC1</td>
<td></td>
</tr>
</tbody>
</table>
4.8 Regulatory relationships about popular TFs

The same method was used to compare and analyze each popular TF gained from P-match method. Firstly, this type of TF consists of six kinds of neighbors (three kinds of precursor nodes, three kinds of successor nodes), including E2F1, E2F3, NFKB1 and YY1. This paper takes E2F1 as an example for detailed analysis.

Table 3 shows E2F1, its predecessors and successors as well as their regulatory relationships. There are six differentially expressed miRNAs target E2F1 that regulate seven differentially expressed miRNAs. Ten miRNAs target E2F1 that regulate 16 miRNAs in the related network. Moreover, 17 miRNAs target E2F1 that regulate 20 miRNAs in the global network. Our study found that eight miRNAs and E2F1 separately constitute three self-adaption relations in Table 3. In the self-adaption relations, E2F1 is not a differentially expressed gene in EWS but hasa-let-7a-1, hasa-let-7a-2, hasa-let-7a-3, hasa-miR-106a and hasa-miR-106b are differentially expressed. It suggests those five miRNAs can indirectly affect expressions of other miRNAs through E2F1. The pathways about E2F1 and the differentially expressed miRNAs indicated that there are important differentially expressed miRNAs in EWS. E2F1 also indirectly influence other TFs by some miRNAs, for example E2F1 regulates hsa-miR-106a that targets RUNX1. Some TFs also indirectly influence E2F3 by some miRNAs, such as NFKB1 regulates hsa-miR-34a that targets E2F1.

Secondly, this type of TF consists of five kinds of neighbors (three kinds of precursor nodes, two kinds of successor nodes), including RUNX1. Thirdly, this type of TF consists of four kinds of neighbors (one kind of precursor node, three kinds of successor nodes), including ZEB1. Fourthly, this type of TF consists of three kinds of neighbors (a kind of precursor node, two kinds of successor nodes, or three kinds of successor nodes, or three kinds of precursor nodes), including RELA, NKX2-5 and E2F2. Fifthly, this type of TF consists of two kinds of neighbors (two kinds of successor nodes), including TCF3 and REL. Sixthly, this type of TF consists of a kind of neighbor (a kind of precursor node), including ATF6 and NF1.

In addition, four TFs (E2F1, E2F3, NFKB1 and ZEB1) and corresponding miRNAs form their self-adaption relationships.

Table 3. Regulatory relations between E2F1 and miRNAs.

<table>
<thead>
<tr>
<th>Differentially expressed network</th>
<th>Related network</th>
<th>Global network</th>
<th>Gene symbol</th>
<th>Differentially expressed network</th>
<th>Related network</th>
<th>Global network</th>
</tr>
</thead>
<tbody>
<tr>
<td>let-7a-1</td>
<td>let-7a-1</td>
<td>let-7a-1</td>
<td>E2F1</td>
<td>let-7a-1</td>
<td>let-7a-1</td>
<td>let-7a-1</td>
</tr>
<tr>
<td>let-7a-2</td>
<td>let-7a-2</td>
<td>let-7a-2</td>
<td></td>
<td>let-7a-2</td>
<td>let-7a-2</td>
<td>let-7a-2</td>
</tr>
<tr>
<td>let-7a-3</td>
<td>let-7a-3</td>
<td>let-7a-3</td>
<td></td>
<td>let-7a-3</td>
<td>let-7a-3</td>
<td>let-7a-3</td>
</tr>
<tr>
<td>miR-106a</td>
<td>miR-106a</td>
<td>miR-106a</td>
<td></td>
<td>miR-106a</td>
<td>miR-106a</td>
<td>miR-106a</td>
</tr>
<tr>
<td>miR-106b</td>
<td>miR-106b</td>
<td>miR-106b</td>
<td>E2F1</td>
<td>miR-106b</td>
<td>miR-106b</td>
<td>miR-106b</td>
</tr>
<tr>
<td>miR-34a</td>
<td>miR-34a</td>
<td>miR-34a</td>
<td></td>
<td>miR-92a-1</td>
<td>miR-92a-1</td>
<td>miR-92a-1</td>
</tr>
<tr>
<td>miR-17</td>
<td>miR-17</td>
<td>miR-17</td>
<td></td>
<td>miR-92a-2</td>
<td>miR-92a-2</td>
<td>1</td>
</tr>
<tr>
<td>miR-20a</td>
<td>miR-20a</td>
<td>miR-20a</td>
<td></td>
<td>miR-92a-2</td>
<td>miR-92a-2</td>
<td>1</td>
</tr>
<tr>
<td>miR-21</td>
<td>miR-21</td>
<td>miR-21</td>
<td></td>
<td>miR-15b</td>
<td>miR-15b</td>
<td>2</td>
</tr>
<tr>
<td>miR-23a</td>
<td>miR-23a</td>
<td>miR-23a</td>
<td></td>
<td>miR-17</td>
<td>miR-17</td>
<td>let-7i</td>
</tr>
</tbody>
</table>
5 DISCUSSION

In this part, we concentrate on the differentially expressed network and related network and give a simple comparison between EWS and other two regulatory networks of Hodgkin's lymphoma (HL) (Zhu, M. 2013.) and Retinoblastoma (Rb) (Li, J. 2013.). It is worth noting that we found significant elements and regulatory relations that are EWS-specific and some elements and regulatory relations that exist in other cancer network but not in EWS in this three network comparison.

Firstly, we compare the networks of EWS and HL. Both of TP53, BCL2 are included in differentially expressed networks of EWS and HL. TP53 forms a self-adaption relationship with hsa-miR-125b-1 and hsa-miR-125b-2 only in the differentially expressed network of EWS. Besides, TP53 regulates hsa-miR-145 which targets FLI1, and regulates hsa-miR-29a, hsa-miR-29b, hsa-miR-29c that target BCL2 and other miRNAs in the differentially expressed network of EWS. In the differentially expressed network of HL, TP53 is only targeted by hsa-miR-125a and hsa-miR-16. The miRNAs regulated by TP53 in above two cancers are much different.

CDKN2A is a significant prognostic factor in a meta analysis of EWS. It is targeted by hsa-miR-125b-1 and hsa-miR-125b-2 in the differentially expressed network of EWS while it doesn’t exist in the differentially expressed network of HL.

Besides, BCL2 is targeted by 13 miRNAs in the differentially expressed network of EWS. It is targeted by 8 miRNAs in the differentially expressed network of HL. Hsa-miR-18a exists in regulatory networks, and hsa-let-7a-1, hsa-let-7a-2, hsa-let-7a-3 target BCL2 in the differentially expressed network of EWS while they target PRDM1 and make self-adaption relationship with MYC in the differentially expressed network of HL. And there are more genes and miRNAs that are not exist in the related network of HL. For example, TGBF1’s mutation has significant corresponding relation with the clinical feature of EWS.

<table>
<thead>
<tr>
<th>miR-126</th>
<th>miR-149*</th>
<th>miR-155</th>
<th>miR-223</th>
<th>miR-330</th>
<th>miR-93</th>
<th>miR-98</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-18a</td>
<td>miR-15b</td>
<td>miR-19a</td>
<td>miR-17</td>
<td>miR-19a</td>
<td>miR-18a</td>
<td>miR-19b</td>
</tr>
<tr>
<td>miR-19a</td>
<td>miR-19b-2</td>
<td>miR-20a</td>
<td>miR-20b</td>
<td>1</td>
<td>miR-19b-1</td>
<td></td>
</tr>
<tr>
<td>miR-20a</td>
<td>miR-20b</td>
<td>miR-25</td>
<td>miR-18b</td>
<td>miR-363</td>
<td>miR-93</td>
<td></td>
</tr>
</tbody>
</table>

Secondly, we compare the networks of EWS and Rb. All the three miRNAs hsa-miR-29a, hsa-miR-29b, hsa-miR-29c exist in the differentially expressed networks of EWS and Rb. hsa-miR-29a, hsa-miR-29b, hsa-miR-29c target DNMT3A, DNMT3B, VEGFA, DNMT1 together or partly in differentially expressed network of Rb, while they target BCL2 together in differentially expressed network of EWS. And there are more genes and miRNAs exist in differentially expressed networks of EWS but not in the differentially expressed networks of Rb, such as hsa-miR-145 and FLI1.

The factors in differentially expressed network and related networks are highly important in research and should be paid much attention to. Different cancer includes different elements and relationships in the three networks we constructed and there must be similarities between similar cancers. We believe that if the differentially expressed data can be regulated to normal state, then cancer may be controlled or even be cured in theory.

6 CONCLUSION

This paper has collected all the experimentally validated genes and miRNAs related to EWS as well as gained predicted TFs using P-match method, and derived differentially expressed network, related network and global network to analyze the regulatory pathways of the differentially expressed elements in EWS. Our study found some important pathways and a network about the development of EWS. Some pathways showed special feature. Thirteen differentially expressed miRNAs (hsa-let-7a-1, hsa-let-7a-2, hsa-let-7a-3, hsa-miR-125b-1, hsa-miR-125b-2, hsa-miR -106a, hsa-miR-106b, hsa-miR-145, hsa-miR-221, hsa-miR-222, hsa-miR-26a-1, hsa-miR-26a-2, hsa-miR-34a) and corresponding genes form 17 self-adaption relationships, mainly including E2F1, MYC, NFkB1, TP53, ESR1, E2F3 and STAT3. Four popular TFs (E2F1, E2F3, NFkB1 and ZEB1) and corresponding miRNAs form their self-adaption relationships. Some TFs were identified by
combining pattern matching and weight matrix approaches in the 1,000-nt promoter region sequences, and were then mapped onto the promoter region of the targets. BCL2, TGFB1, NFKB1, FLI1 and E2F1 should be paid more attention to in future of EWS. The TFs predicted from this method may reveal key relationships between the differentially expressed miRNAs and TFs.

In the following work, our study will consider the interaction of proteins and regulatory pattern (up regulation and down regulation) into our network. We will derive a more comprehensive and extensive network about EWS. Consequently, it may contribute to the prognosis, diagnoses and therapy of EWS.

7 CONFLICTS OF INTEREST

There is no conflict of interest.

8 ACKNOWLEDGEMENT

This work was supported by the grants from National Natural Science Foundation of China (No.60973091) and Science and Technology Development Plan of Jilin Province (No.20130101166JC).

REFERENCES